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COMPLEMENT FIXATION OF STREPTOCOCCI

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Streptococci have been suspected to be the etiologic factor in so many different diseases that for years investigators have tried to determine a satisfactory method by which the various strains could be grouped. Classifications have been made on the basis of (1) morphology, (2) disease source, (3) pathogenicity for animals, (4) power to lake blood, (5) carbohydrate fermentation, (6) immunity tests, especially agglutination and complement fixation, and (7) various combinations of these methods. Comparatively few attempts have been made to classify the streptococcus strains by the complement fixation test. Therefore, it was thought that it might be of value to attempt a classification by the fixation reaction of strains isolated from a number of definite diseases. The following is a brief résumé of the literature on streptococcus complement fixation previously reported.

REVIEW OF LITERATURE

As early as 1904 Besredka[1] inoculated a horse with streptococci, obtaining an immune serum which was specific for that one strain. However, when Besredka and Dopter[2] applied the method to serum from scarlet fever patients, they had no positive results. Schleisser[3] and Sommerfeld[4] also found no positive complement fixation in scarlet fever cases.

Livierato,[5] however, tested the serums of 18 cases of scarlet fever with the following antigens: streptococcus isolated from scarlet fever cases, streptococcus of erysipelas, staphylococcus, diplococci, and typhoid bacillus. All the serums gave positive results with the scarlet fever streptococcus antigen and negative results with the other antigens. Uffenheimer[6] also found that there was specific complement fixation between scarlet fever serum and scarlet fever streptococcus antigen and that the fixation was strongest on the tenth day.

Foix and Mallein[7] obtained specific complement fixation with scarlet fever serum and scarlet fever streptococcus antigen; and in a later series of experiments[8] they observed that serum from 10 of 12 cases of scarlet fever fixed

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[1] Ann. de l'Inst. Pasteur, 1904, 18, p. 363.

[2] Ibid., p. 373.

[3] Wien. klin. Wchnschr., 1908, 21, p. 1375.

[4] Arch. f. Kinderh., 1909, 50, p. 38.

[5] Gazz. d. osp., 1907, 28, p. 835.

[6] München. Med. Wchnschr., 1909, 56, p. 2471.

[7] Presse méd., 1907, 15, p. 777.

[8] Presse méd., 1910, 18, p. 215.

9 strains of streptococcus which had been isolated from the throat of scarlet fever patients. Serum of patients with erysipelas and puerperal fever used as control gave negative results. Foix and Mallein concluded that the streptococcus of scarlet fever was a specialized strain.

Castex[9] used serum from persons having erysipelas, scarlet fever, abscess, puerperal fever, pleurisy, and arthritis with streptococcus antigen and obtained positive results with everyone except 3 scarlet fever serums. He considered, therefore, that there were no specific antibodies produced by these various streptococcus strains.

Swift and Thro[10] immunized 5 rabbits with different strains of streptococci, and, while the complement fixation was not absolutely specific, they considered that it might be a means of studying specific streptococcus infections.

Kolmer[11] tested serum from 107 scarlet fever patients, using as antigen a streptococcus isolated from scarlet fever, and secured 11.2% fixations. He also immunized several animals with streptococci from scarlet fever cases and one, as a control, with a streptococcus isolated from a nonscarlatinal tonsillitis. He tested each immune serum against all antigens used in the inoculations and from his results deduced that a strain of streptococci produced a specific antibody up to a certain limit; that is, with low dilutions the specific nature of the antibody was not always observed, but in high dilutions it was.

Major[12] reported a case of endocarditis lenta in which the serum contained antibodies for a Streptococcus viridans which had been previously isolated from the patient. The serum, however, also fixed a hemolytic streptococcus antigen though not a pneumococcus antigen.

Hastings studied the complement fixation of bacteria from arthritis deformans. Twelve of the 24 serums tested gave positive fixation with a Streptococcus viridans antigen. In a later series of experiments[14] he used a polyvalent streptococcus antigen for fixation tests in an effort to detect S. viridans infections. He decided that the serums were so poor in fixing substances and the antigens so poor in fixable substances that the method would not be available for clinical work.

Floyd and Wolbach[15] attempted to correlate streptococcus complement fixations with a classification of streptococcus based on cultural characteristics rather than on the disease from which the strains had been isolated. After performing cross fixation experiments with 7 strains, they concluded that there was a marked degree of specificity between antigens of a given sugar group and their corresponding antiserums.

Kinsella,[16] from the results of fixation tests in a study of subacute streptococcus endocarditis, concluded that the strains causing the disease could be roughly grouped in three classes. In his experiments this classification had no apparent relation to the grouping based on fermentation reactions.

Swift and Kinsella[17] obtained practically the same results in a similar study of the strains isolated in acute rheumatism. Kinsella and Swift[18] then classified nonhemolytic streptococci as a whole, on the basis of complement fixation, and considered that they could be placed in 3 groups. In looking over their fixation results, they observed as follows: "There was an inverse ratio between the fixing capacity on the part of a serum and the capacity to be fixed on the part of the corresponding antigen." They therefore made 3 groups: a first group in which the antigens used in cross-fixation tests fixed extensively and the corresponding antiserums slightly; a second group in which the serums fixed extensively and the corresponding antigens slightly; and a third group in which there were intermediate reactions. They considered that this classification might be explained by the possible presence of two diverse elements in the group and that some strains entirely consisted of one element, some of the other, and other strains of both.

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[9] Presse méd., 1909, 17, p. 324.

[10] Arch. Int. Med., 1911, 7, p. 24.

[11] Arch. Int. Med., 1912, 9, p. 220.

[12] Bull. Johns Hopkins Hosp., 1912, 23, p. 326.

[13] Jour. Am. Med. Assn., 1912, 60, p. 1208.

[14] Jour. Exper. Med., 1914, 20, p. 72.

[15] Jour. Med. Research, 1913, 29, p. 493.

[16] Arch. Int. Med., 1917, 19, p. 367.

[17] Ibid., p. 381.

[18] Jour. Exper. Med., 1917, 25, p. 877.

Aschner[19] by complement fixation tests found that the serums of patients with subacute streptococcus endocarditis give strongly positive fixation with a homologous antigen, a positive with a polyvalent antigen of strains from other endocarditis patients, and a negative with a mixed antigen of similar strains isolated from the throat and local lesions.

TECHNIC

Cultures.—Sixty-five strains of streptococci isolated from definite diseases were used in these tests; 28 were hemolytic and 37 nonhemolytic. Two strains of pneumococci were used as controls. Stock cultures were grown on human blood agar. Cultures for inoculations and antigens were 18-24 hour growths in ascitic dextrose broth.

Antigens.—Bacteria from each culture were removed by centrifugation, washed twice, and suspended in sterile normal salt solution in such quantity that a faintly cloudy, smooth suspension resulted. These suspensions were used for inoculation. For antigens, similar suspensions were heated at 56 C. for 30 minutes.

Immunization.—Twenty-eight rabbits, 3-6 months old, were immunized; 12 against hemolytic strains of streptococcus, 14 against nonhemolytic strains of streptococcus, and 2 against pneumococci. Before immunization the serum of each rabbit was tested with the following antigens: luetic liver extract, ascitic dextrose broth, and the strain selected for inoculation. Only rabbits whose serum gave complete hemolysis with these antigens were used for inoculations. In each rabbit immunization was obtained by intravenous inoculation of the living suspension of the desired strain, at 3 or 4 day intervals, the dosage gradually increasing from 0.25-2 c.c. When 0.02 c.c. of a rabbit's serum gave complete inhibition of hemolysis with $\frac{1}{120}$ of the anticomplementary unit of the corresponding antigen, the rabbit was considered immune. This usually occurred after 7-10 inoculations. Each immune rabbit was bled from the heart. The clear serums were sealed in ampules, inactivated at 56 C. for 30 minutes, and stored in the refrigerator. Immunity was maintained in the rabbits that survived bleeding by weekly subcutaneous inoculation of 2 c.c. of the living bacterial suspension.

Fixation Technic.—The antisheep-rabbit system was used in one-tenth the volume of the classic Wassermann. Each day the anticomplementary unit of antigen was determined, and the hemolytic system was standardized by an amboceptor titration. In the test, the serum remained a constant 0.02 c.c.; the antigen varied from $\frac{1}{4}$-$\frac{1}{120}$ of the anticomplementary unit. Fresh guinea-pig serum 0.1 c.c. of a 1:10 dilution (2 units) was used as complement. Serum, antigen, and complement were incubated at 37 C. for 1 hour. Two units of previously titrated antisheep amboceptor and 0.1 c.c. of sheep's corpuscles in 5% suspension were added to each tube, and the whole incubated for 30 minutes. The customary controls (antigen, serum, and hemolytic) were set up with each test.

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[19] Jour. Infect. Dis., 1917, 21, p. 409.

Each immune serum was tested with each of the streptococcus antigens and with the following control antigens; two pneumococcus, luetic liver, and ascitic dextrose broth as antigen.

COMPLEMENT FIXATION OF STREPTOCOCCUS GROUPED ACCORDING TO HEMOLYSIS OR NONHEMOLYSIS

From the reports on agglutination of streptococcus strains, it seemed probable that the fixation reaction of hemolytic and nonhemolytic strains would be quite different and that a primary division of strains might be made on the basis of hemolysis or nonhemolysis. The hemolytic or nonhemolytic property of a strain was determined by an examination of human blood-agar block plates of each streptococcus. Contrary to expectation, there was marked cross fixation between the two groups. An examination of the results indicated that hemolytic antiserums apparently fixed antigens of hemolytic strains to a greater degree than they did antigens of nonhemolytic strains. The hemolytic antiserums also gave less positive fixation with nonhemolytic antigens than did the nonhemolytic antiserums. It was quite obvious, though, that nonhemolytic antiserums fixed antigens of hemolytic strains at least as well as they did antigens of nonhemolytic streptococci. The percentage of positive fixation of each nonhemolytic serum was then computed with both nonhemolytic and hemolytic antigens (Table 1). The 3 and 4 plus positives were computed together, the 1 and 2 plus positives together, and lastly the total positives (Fig. 1). When these percentages were plotted, it was seen that the contours of the curves of the three were similar. Therefore, the curve of the total fixations only was plotted (Fig. 1). In each case the nonhemolytic antiserum gave a higher percentage of positive fixation with hemolytic antigens than with nonhemolytic antigens, namely, the total fixation with nonhemolytic antigens was 58.2%, while with the hemolytic antigen it was 68.6%.

The percentage of positive fixation of hemolytic immune serums with hemolytic and nonhemolytic antigens was computed in the same way (Table 1). There was the same relation between the curves of the total positives, strongly positives, and faintly positives as was noted with the nonhemolytic antiserums (Fig. 2). Here, however, the percentage of positive fixation was slightly greater with the homologous group antigens than with the heterologous group antigens, namely, the total positive fixation with hemolytic antigens was 61.9% and with nonhemolytic 48.4%. From these results it appeared that the nonhemolytic antiserums were less specific in their action than the hemolytic.

TABLE 1

PERCENTAGE OF FIXATION OF ANTISERUM WITH HEMOLYTIC AND NONHEMOLYTIC ANTIGENS

Antiserums	Hemolytic Antigens		Nonhemolytic Antigens	
	Number of Tests	Percentage of Fixation	Number of Tests	Percentage of Fixation
Hemolytic				
A6	11	54	16	62
A8	28	71	37	62
A10	28	53	37	46
D5	28	60	36	33
D7	28	71	36	47
E1	28	75	37	62
E2	28	18	37	16
E3	28	75	36	41
L1	2	50	5	83
L4	17	41	26	42
M1	28	79	37	65
S1	22	81	33	70
Total	276	62	373	48
Nonhemolytic				
A1	15	40	24	50
A2	28	82	37	78
A7–1	14	78	24	66
A7–2	28	78	37	76
A11	23	65	32	34
B1	28	85	36	69
B2–1	10	30	12	33
B2–2	28	67	36	74
G1	28	78	36	61
G2	28	78	36	33
L13	28	53	36	44
L14	28	96	36	86
M14	28	39	37	48
T1	27	58	36	38
Total	341	68	455	58

COMPLEMENT FIXATION OF STREPTOCOCCI GROUPED ACCORDING TO DISEASE

Antigens were made of each of the 65 strains and an effort was made to immunize at least 2 rabbits in each disease group.

GROUP A (*Acute Poliomyelitis*).—Eleven antigens and 8 antiserums were employed in this group. The results of the cross complement fixation tests within the group and with several control antigens has been previously reported.[20] At that stage in the tests there appeared to be almost complete group specificity. Since that report, though, the immune serums have been tested with the antigens of the other disease

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[20] Mathers and Howell: Jour. Infect. Dis., 1917, 21, 292.

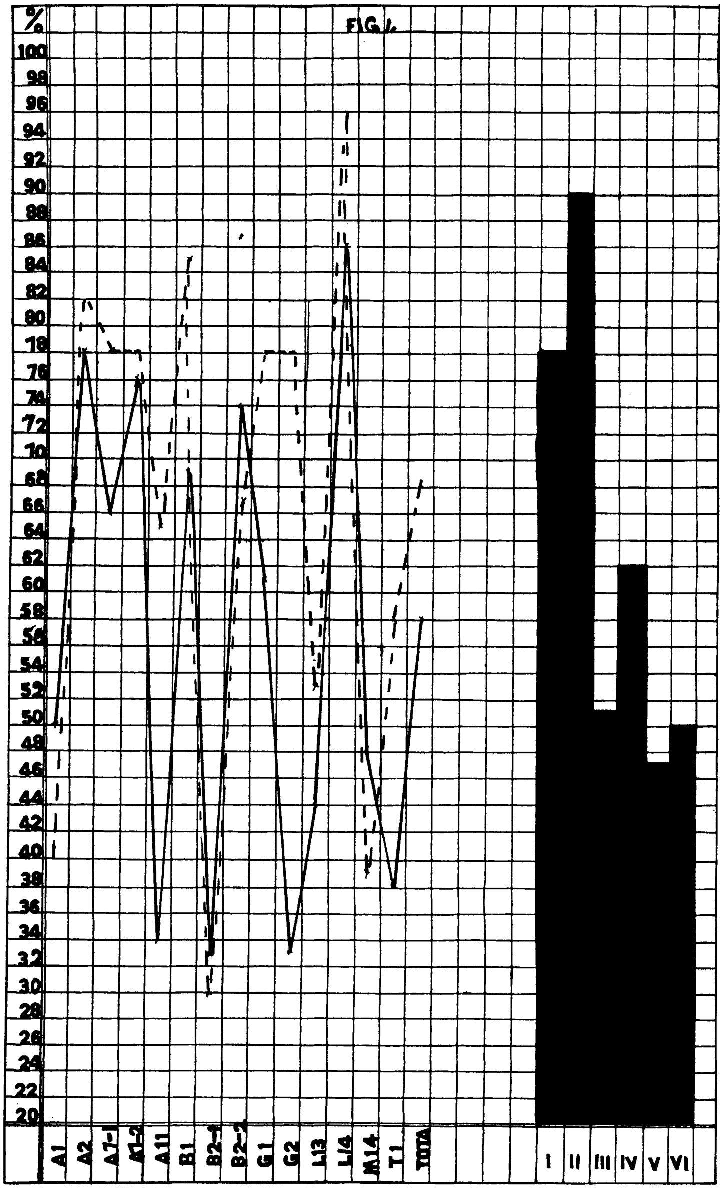

Fig. 1.—Solid line = percentage of fixation of nonhemolytic serums with nonhemolytic antigens. Broken line = percentage of fixation of nonhemolytic serums with hemolytic antigens.

I 455 nonhemolytic antigens, 265 total fixations = 58%
II 341 hemolytic antigens, 234 total fixations = 68%
III 455 nonhemolytic antigens, 142 fixations (1+, 2+) = 31 %
IV 341 hemolytic antigens, 131 fixations (1+, 2+) = 38%
V 455 nonhemolytic antigens, 123 fixations (3+, 4+) = 27%
VI 341 hemolytic antigens, 103 fixations (3+, 4+) = 30%

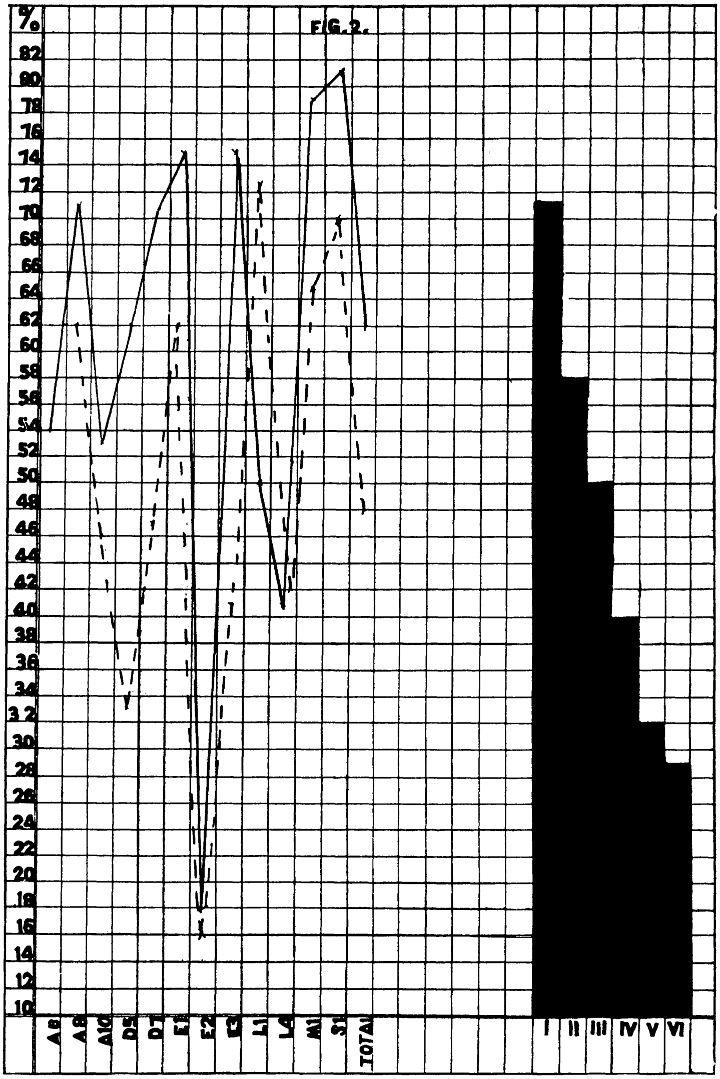

Fig. 2.—Solid line = percentage of fixation of hemolytic antiserums with hemolytic antigens. Broken line = percentage of fixation of hemolytic antiserums with nonhemolytic antigens.

I 277 hemolytic antigens, 172 total fixations = 61%
II 373 nonhemolytic antigens, 181 total fixations = 48%
III 277 hemolytic antigens, 110 fixations (1+, 2+) = 40%
IV 373 nonhemolytic antigens, 111 fixations (1+, 2+) = 30%
V 277 hemolytic antigens, 62 fixations (3+, 4+) = 22%
VI 373 nonhemolytic antigens, 70 fixations (3+, 4+) = 19%

groups with the result that they gave considerable scattered fixation. Two rabbits in this group were immunized against the same strain (A7), and it was interesting to note that the antiserums (A7-1 and A7-2) did not give parallel complement fixation reactions either in the homologous or heterologous groups.

GROUP B (*Endocarditis*).—Six strains were isolated from the blood of patients with endocarditis. Two rabbits were immunized against strains B1 and B2, respectively. Rabbit B2 died after immunization and another rabbit was inoculated with the B2 strain. The complement reactions of the antiserums B2-1 and B2-2 were fairly similar. The 3 immune serums gave strong fixation with the endocarditis antigens, but fixed almost equally well the antigens of the sepsis and measles groups. There was also considerable cross fixation with the other streptococcus group antigens. The antiserums gave fixation, ranging from + to ++++ with the 2 pneumococci antigens, and B2-2 gave a weakly positive reaction with luetic liver antigen. The serum would seem, therefore, to give nonspecific reactions.

GROUP D (*Sepsis*).—The strains used in this group were isolated from the blood of patients with severe sepsis: puerperal sepsis (D1, D2, D7); secondary to empyema (D4, D6); post-operative (D3); secondary to brain abscess (D5); following acute mastoiditis (D8); from meningitis (D9). Strains D5 and D7 were used for immunization. There was no apparent group fixation. Antigens from the same disease did not have similar fixative power with the various antiserums.

GROUP E (*Erysipelas*).—The three strains were isolated from erysipelas, E1 from a bleb and E2 and E3 from blood cultures. Antigens and immune serums of each strain were used in the fixation tests. Considering this group alone, the group fixation was apparently specific, but examining the chart as a whole, the usual cross fixations with other group antigens were observed.

GROUP G (*Acute Articular Rheumatism*).—The two strains were isolated from the blood of patients with acute articular rheumatism. These strains were obtained from Swift and Kinsella.[17] The results of the group fixation corresponded to those obtained by them in that the antiserum G1 fixed with antigen G1, but not with antigen G2 and vice versa. There was considerable cross fixation with the other streptococcus groups. The cross fixation with the endocarditis group composed of nonhemolytic strains that were similar culturally, was very slight, while that with the sepsis group composed mostly of hemolytic strains, was especially strong. The fixation with the pneumococcus antigen was strongly positive.

GROUP L (*Upper Respiratory Tract Infections*).—This rather general group included strains isolated from the nasal discharge in acute and chronic coryza (L1-L5), from sputum in la grippe (L5-L8), and from the ulcers in tonsillitis (L8-L14). The tonsillitis antiserums (L13, L14) fixed the majority of the tonsillitis antigens; with that exception, there was no group specificity.

GROUP M (*Measles*). — In this group, 13 strains were used as antigens. Eleven strains were isolated from measles cases and 2 (M10, M13) from German measles. Two antiserums (M1, M14) were used in the test. M1, a hemolytic strain, was isolated from the throat of a measles patient, and M14, a nonhemolytic strain, from the blood of a measles patient just before the appearance of the rash. The fixation of the 2 immune serums were quite different. M1 fixed all the acute poliomyelitis antigens while M14 gave only a slight fixation with 2 antigens in that group. Both antiserums fixed a number of antigens in the measles and upper respiratory tract groups, but frequently not the same antigen. There was fixation with the pneumococcus antigens, slight with M1, strong with M14.

GROUP S (*Scarlet Fever*). — There was only one scarlet fever antigen and antiserum. The strain was isolated from the blood of a scarlet fever patient. It was a very virulent organism and 2 rabbits died in the course of immunization. The third rabbit did not develop an immune serum of high titer for the strain. The fixation with antigens of the sepsis group was more strongly positive than with the homologous antigen. There was cross fixation with other group antigens, especially with endocarditis and pneumococcus strains.

GROUP T (*Thrombosis*).—This was a nonhemolytic streptococcus from the blood of a patient with thrombosis. Only one rabbit was immunized before the strain was lost.

GROUP X (*Miscellaneous*). — This group contained miscellaneous strains: 2 from lung abscesses, 1 from a tooth abscess, 1 from the blood of the umbilical cord, and 1 from spinal fluid in meningitis. They were used as antigens only.

GROUP P (*Pneumonia*). — Two rabbits were immunized with 2 strains of pneumococcus as a control group. Unfortunately, both antiserums were probably nonspecific since they had a high percentage of

Rabbit Anti-serums	A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	A11	B1	B2	B3	B4	B5	B6	D1	D2	D3	D4	D5	D6	D7	D8	D9	E1	E2
A1	4	3	0	3	4	2	2	3	4	4	4		0														0	0
A2	3	4	4	3	4	3	3	4	4	4	3	0	2	4	4	2	2	2	2	2	0	3	2	0	3	3	3	2
A6	1	4	0	2	3	4	4	4	2	4	0	1	1	0													1	0
A7-1	0	3	1	3	1	1	4	1	1	2	3	3	2	2	4	0	2								2	4	3	2
A7-2	3	2	3	2	3	4	4	0	1	3	1	3	0	3	0	1	1	1	3	1	0	3	3	0	2	4	3	1
A8	1	4	0	3	3	1	4	4	2	4	0	2	1	3	4	0	0	0	3	1	0	2	3	2	0	2	4	1
A10	3	4	2	2	0	1	4	4	2	4	0	1	1	0	1	0	0	0	2	0	1	2	0	0	0	4	0	0
A11	4	4	0	2	4	2	4	4	4	2	4	2	1	0	0	0	0	2	3	0	2	0	2	0	2	0	2	1
B1	2	4	1	3	2	1	0	2	2	3	2	4	4	0	4	4	4	3	2	3	3	3	2	3	3	4	1	1
B2-1	0	0	0	0	0	0	0	0	0	0	0	2	4	2	0	0	0										0	1
B2-2	1	4	1	2	0	1	0	0	0	2	1	2	4	2	3	4	4	2	3	2	4	1	3	2	3	4	0	1
D5	0	2	0	0	0	1	2	0	0	0	2	0	0	0	0	1	0	0	1	0	1	4	1	0	2	1	2	0
D7	0	0	0	0	0	1	0	0	1	0	2	0	0	0	0	0	0	1	4	1	2	4	3	4	2	0	3	2
E1	1	0	1	1	3	0	0	2	2	0	2	1	1	4	0	0	0	1	4	0	1	2	2	1	2	1	4	4
E2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	2	3
E3	0	0	0	1	0	1	0	0	2	1	1	0	0	0	0	0	0	3	4	1	3	3	4	0	0	0	2	2
G1	0	1	0	2	0	1	0	0	4	3	3	2	2	0	1	2	0	2	2	0	3	4	3	3	2	1	1	1
G2	0	2	0	3	0	0	0	0	2	2	0	0	0	0	0	0	0	1	4	2	3	2	3	3	1	1	0	1
L1	1	1	1			0						3	1	0														
L4	1	1	1	0	0	0	0	0	0	0	2	3	1	0	0	0	0								3		4	1
L13	0	1	0	2	0	1	0	0	0	0	2	1	0	0	0	1	0	0	1	3	3	4	1	2	1	0	1	1
L14	0	3	0	3	2	1	1	2	3	3	2	4	3	4	4	4	4	2	3	3	3	4	2	2	2	3	4	4
M1	3	3	4	4	3	3	4	2	4	4	2	2	2	0	0	0	0	3	2	1	2	0	3	1	2	1	0	0
M14	0	0	0	0	0	1	0	1	0	0	2	0	0	0	1	1	0	2	2	0	0	0	0	0	0	2	0	0
S1	1	1	0	0	2	1	0	2	2	0	2	3	1	1	4	4	4	3	4	4	3	4	4	3	3	3	0	1
T1	1	1	1	0	2	1	0	0	0	2	0	0	1	0	0	0	0	0	3	0	1	2	0	0	4	3	1	0
P1	3	2	3	3	4	4	4	3	3	4	0	1	1	4	3	0	2	1	1	0	2	2	0	1	2	3	1	0
P2	2	2	0	0	0	2	2	2	2	2	4	3	0	0	3	0	0	0	4	4	3	3	2	4	4	4	1	0
Normal	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

0 = complete hemolysis.
1 = inhibition of hemolysis in 1/4–1/8 of anticomplementary unit.
2 = inhibition of hemolysis in 1/16 of anticomplementary unit.
3 = inhibition of hemolysis in 1/32–1/64 of anticomplementary unit.
4 = inhibition of hemolysis in 1/128, etc., of anticomplementary unit.

TABLE 2

COMPLEMENT FIXATION TESTS

Streptococcus Antigens																																		
E1	E2	E3	G1	G2	L1	L2	L3	L4	L5	L6	L7	L8	L9	L10	L11	L12	L13	L14	M1	M2	M4	M5	M6	M7	M8	M9	M11	M12	M14	M10	M13	S1	T1	X1
0	0	0	0	0		0		0						0	1				0	0	0	0	0	0	0	1	4	0		0		0	2	3
3	2	2	4	4	0	0	4	3	2	2	2	4	4	0	4	3	4	4	1	2	0	4	0	0	0	1	2	0	2	2	1	2	2	0
1	0	0	3	3		1		0						0					0	0												1	0	
3	2	0	3	3		0	1	3						3	0			0	0	0	0	4										1	4	
3	1	1	2	2	4	2	1	0	0	1	2	4	4	3	0	4	4	0	1	1	0	4	0	0	0	1	2	0	2	1	2	1	3	2
4	1	0	0	3	0	2	1	2	3	0	2	3	3	0	4	0	4	4	2	0	0	2	0	0	0	0	3	2	0	0	2	1	3	1
0	0	0	3	3	0	2	0	0	1	0	1	1	0	0	3	0	2	2	1	0	0	0	0	0	0	2	1	0	0	3	0	0	0	0
2	1	0	0	0		0		0						0	0			0	0	0	0	0	1	0	0	0	3	0	0	0	0	0	0	
1	1	0	1	0	3	0	3	4	0	2	0	2	2	2	0	3	3	0	1	0	0	4	2	2	0	3	3	0	2	2	2	0		1
0	1	0						0											1													1	0	
0	1	0	0	0	0	1	3	4	0	1	0	2	2	1	4	2	4	3	2	0	0	4	0	2	0	4	3	0	1	2	3	0		1
2	0	1	0	0	1	1	1	0	2	0	3	0	0	1	0	0	4	0	2	0	0	1	0	1	0	0	0	1	1	0	0	2		0
3	2	1	2	0	0	2	2	3	2	0	2	3	4	2	1	1	4	2	0	0	3	0	0	0	2	1	1	2	3	0	3	3		0
4	4	4	0	0	3	2	4	2	2	1	3	1	2	0	2	1	2	0	1	0	1	2	0	0	0	2	2	0	0	0	1	1	4	2
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					4																													
4	1	0				0	0	4	2	2	0	0	0	0	2			3	0	2	0	0										1	3	0
1	1	2	0	0	0	0	4	0	0	0	0	0	1	0	2	4	4	3	0	0	0	0	0	2	2	1	2	2	0	0	4	2		0
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0	1	2	1	4	1	2	1	4						1	4			2	2	0	0	4	1	0	2	0	0	4	0	0	0	3	3	
1	0	0	0	0		0	1	3	0	1	1	1	0	0	4	1	3	0	0	0	4	0	0	0	0	2	3	0	0	1	0	1	4	
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0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

L11	L12	L13	L14	M1	M2	M4	M5	M6	M7	M8	M9	M11	M12	M14	M10	M13	S1	T1	X1	X2	X3	X4	X5	P1	P2	Luetic Liver Extract	Ascitic Dextrose Broth
1				0	0	0	0	0	0	0	1	4	0		0		0	2	3	2	3	2	0	0	0	0	0
4	3	4	4	1	2	0	4	0	0	0	1	2	0	2	2	1	2	2	0	2	1	3	0	0	0	0	0
				0	0												1	0					0	0	0	0	0
0			0	0	0	0	4										1	4		0			0	0	0	0	0
0	4	4	0	1	1	0	4	0	0	0	1	2	0	2	1	2	1	3	2	4	3	2	0	1	0	0	0
4	0	4	4	2	0	0	2	0	0	0	0	3	2	0	0	2	1	3	1	4	1	2	0	0	0	0	0
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0	3	3	0	1	0	0	4	2	2	0	3	3	0	2	2	2	0		1	4	2	2	0	1	4	0	0
				1													1	0				2		2	2	0	0
4	2	4	3	2	0	0	4	0	2	0	4	3	0	1	2	3	0		1	4	0	1	2	2	3	1	0
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1	1	4	2	0	0	3	0	0	0	2	1	1	2	3	0	3	3		0	3	0	2	0	4	3	0	0
2	1	2	0	1	0	1	2	0	0	0	2	2	0	0	0	1	1	4	2	1	2	2	0	0	1	0	0
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2	4	4	3	1	0	0	0	0	1	2	1	2	3	2	1	2	2		2	4	2	3	0	3	1	0	0
2	4	4	3	0	0	0	0	0	1	2	1	2	2	0	0	3	2		3	4	4	3	0	4	4	0	0
4	0	3	1	1	0	0	1	0	0	2	0	2	0	0	0	0	2		0	3	4	3	0	3	2	0	0
																								2	0	0	0
2			3	0	2	0	0										1	3	0	1	0	0	0	1	0	0	0
2	4	4	3	0	0	0	0	0	2	2	1	2	2	0	0	4	2		0	4	0	3	0	3	2	0	0
4	4	4	4	1	2	4	1	0	3	4	4	2	2	1	0	2	3		0	3	3	3	0	2	2	0	0
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4			2	2	0	0	4	1	0	2	0	0	4	0	0	0	3	3		1		0	4	3	3	0	0
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0	4	2	0	0	0	3	0	0	0	0	1	3	3	0	0	2	0	0	3	2	2	4	0	2	4	1	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

fixation with practically all groups and also gave a weak fixation with luetic liver antigen.

A normal rabbit serum did not inhibit hemolysis with any antigen. The results of the fixation test appear in Table 2.

The percentage of positive fixation of the serums in each group was computed for the homologous group antigen and for the heterologous group antigens (Table 3) and the percentages plotted (Fig. 3). In

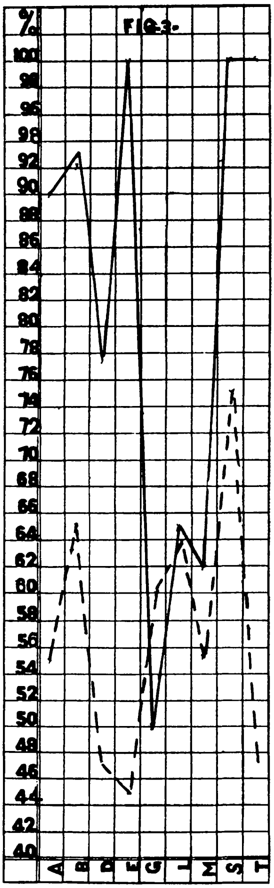

Fig. 3.—Solid line = percentage of fixation of disease group antiserums with homologous antigens. Broken line = percentage of fixation of disease group antiserums with heterologous antigens.

A = Anterior poliomyelitis strains.
B = Endocarditis strains.
D = Sepsis strains.
E = Erysipelas strains.
G = Arthritis strains.
L = Upper respiratory tract infection strains.
M = Measles strains.
S = Scarlet fever strains.
T = Thrombosis strains.

every group except one (Group G) the percentage of positive fixation was greater with the homologous antigens than with the heterologous antigens, the more definite disease groups showing a greater difference between the 2 percentages.

There are too few immune serums and antigens in each group to justify drawing any definite conclusions, but the data suggest that there is no clear cut group specificity.

TABLE 3

PERCENTAGE OF FIXATION OF DISEASE GROUP ANTISERUMS WITH HOMOLOGOUS AND HETEROLOGOUS GROUP ANTIGENS

Antiserums		Homologous Antigens		Heterologous Antigens	
Number	Group	Number of Tests	Percentage of Fixation	Number of Tests	Percentage of Fixation
8	A (Acute poliomyelitis)	88	90	331	55.5
3	B (Endocarditis)	15	92	134	64.9
2	D (Sepsis	18	77	110	47.2
3	E (Erysipelas)	9	100	185	44.8
2	G (Acute rheumatic fever)	4	50	122	60
4	L (Upper respiratory tract infections)	40	65	136	64
2	M (Measles)	26	62.5	104	55.7
1	S (Scarlet fever)	1	100	55	75
1	T (Thrombosis)	1	100	62	47.3

COMPLEMENT FIXATION OF STREPTOCOCCI GROUPED ACCORDING TO CARBOHYDRATE FERMENTATION

The mediums used for the fermentation tests were sugar-free broth (peptone 1%, salt 0.5%, Liebig's beef extract 0.25%) plus 1% of the various carbohydrates. The reaction was titrated to plus 1.0 with phenolphthalein as an indicator. The following carbohydrates were used: dextrose, lactose, saccharose, maltose, salicin, inulin, raffinose, and mannit. All cultures were inoculated into one kind of sugar broth medium on the same day. Also control tubes of the same medium were used. The cultures were incubated at 37.5 C. for 8 days, and the readings were made at the end of that time. Five c.c. of the broth in each tube was thoroughly mixed in a flask with 45 c.c. of freshly boiled distilled water and titrated in the cold against N/20 NaOH, using phenolphthalein as an indicator. The average titration of the 2 control tubes was noted, and, arbitrarily, everything above this point was considered positive fermentation.

The streptococci were then grouped according to both Holman's[21] and Andrewes and Horder's[22] classifications. However, so many of

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[21] Jour. Med. Research, 1916, 34, p. 377.

[22] Lancet, 1906, 2, pp. 708, 775, 852.

TABLE 4

STREPTOCOCCUS STRAINS GROUNED ACCORDING TO CARBOHYDRATE CLASSIFICATION

Group	Strains
Pyogenes..............	A10, D6, D7, D8, D9, E1, E2, L4, L7, L8, L10, L12, M1, M7, M10, S1, X2
Mitis..................	A2, A4, A5, B2, B3, B4, B5, B6, D1, G1, L2, L3, L6, M4, M5, M11, M13
Fecalis.................	A1, B1, G2, L9, L13, L14, M6, X4, X5
Infrequens.............	A6, A8, A9, D3, D4, D5, L1, L5, M8
Ignavus................	A3, A7, A11, M12
Salivarius.............	M9, M14, T1, X1
Anginosus.............	D2, X3
Hemolyticus I.........	E3
Equinus................	M2

TABLE 5

PERCENTAGE OF FIXATION OF ANTISERUMS AND ANTIGENS GROUPED ACCORDING TO CARBOHYDRATE CLASSIFICATION

Antiserums		S. Pyogenes		S. Mitis		S. Fecalis		S. Infrequens	
No.	Group	No. Tests	Per Cent. Positive	No. Tests	Per Cent. Positive	No. Tests	Per Cent. Positive	No. Tests	Per Cent. Positive
7	Pyogenes..........	111	60	114	47	70	57	57	60
4	Mitis..............	48	80	56	74	33	72	30	53
5	Fecalis............	78	70	76	60	48	56	40	77
4	Infrequens.........	41	61	51	57	25	65	23	65
3	Ignavus...........	41	70	45	66	24	46	19	63
2	Salivarius.........	34	56	34	44	20	40	17	35
1	Hemolyticus.......	17	71	17	30	10	25	9	77

the strains were variants of the main groups of Andrewes and Horder that their classification was discarded and Holman's alone used. The majority of the strains were in the following groups: S. pyogenes, S. mitis and S. fecalis (Table 4).

The immune serums and the antigens were then grouped according to this sugar classification, and the complement fixation reactions of each serum and antigen noted. The percentage of positive complement fixation of the immune serums of each sugar group, with the homologous and heterologous sugar group antigen, was computed. The results appear in Table 5. Although there are too few strains in some groups to draw definite conclusions, taken as a whole the table indicates that there is little or no sugar group specificity. It is interesting to note that group S. pyogenes shows a slight degree of specificity, while S. mitis and S. fecalis antiserums have a higher percentage of fixation with heterologous antigens than with their homologous group antigens — a result similar to that given by the hemolytic and nonhemolytic antiserums in the classification based on hemolysis and nonhemolysis. The results differ markedly from that of Floyd and

Wolbach,[15] who considered that complement fixation tests while not absolutely specific indicated a marked degree of specificity between strains of a given sugar group and their corresponding antiserums.

COMPLEMENT FIXATION OF STREPTOCOCCI GROUPED ACCORDING TO OTHER METHODS

The percentage of fixative power of each immune serum and the percentage of fixative power of its corresponding antigen was computed (Table 6) and a curve plotted (Fig. 4) in order to see whether

TABLE 5

PERCENTAGE OF FIXATION OF ANTISERUMS AND ANTIGENS GROUPED ACCORDING TO CARBOHYDRATE CLASSIFICATION

S. Ignavus		S. Salivarius		S. Anginosus		S. Hemolyticus I		S. Equinus	
No. Tests	Per Cent. Positive	No. Tests	Per Cent. Positive	No. Tests	Per Cent. Positive	No. Tests	Per Cent. Positive	No. Tests	Per Cent. Positive
28	52	23	56	12	64	7	71	7	17
15	47	11	86	6	83	4	50	3	33
20	45	15	60	9	66	5	60	5	20
12	58	8	37	5	80	3	33	3	0
11	73	8	62	4	75	3	33	3	33
8	33	7	57	4	33	2	0	2	0
4	50	3	100	2	100	1	100	1	0

TABLE 6

PERCENTAGE OF FIXATIVE POWER OF ANTISERUMS AND ANTIGENS

Strain	Antiserum Fixation		Antigen Fixation	
	Number of Times Used	Percentage of Fixation	Number of Times Used	Percentage of Fixation
A1	41	38	26	58
A2	65	80	26	77
A6	27	61	26	77
A7-1	38	71	25	42
A7-2	65	77	25	42
A8	65	66	26	54
A10	65	49	26	61
A11	55	40	26	69
B1	64	78	26	68
B2-1	22	32	26	65
B2-2	64	72	26	65
D5	64	45	20	55
D7	64	56	20	55
E1	65	69	26	68
E2	65	17	26	72
E3	64	56	26	52
G1	64	69	26	50
G2	64	60	26	48
L1	8	86	19	63
L4	43	58	26	56
L13	64	48	18	100
L14	64	91	19	54
M1	65	71	26	64
M14	65	51	20	50
S1	36	75	26	76
T1	63	48	16	62

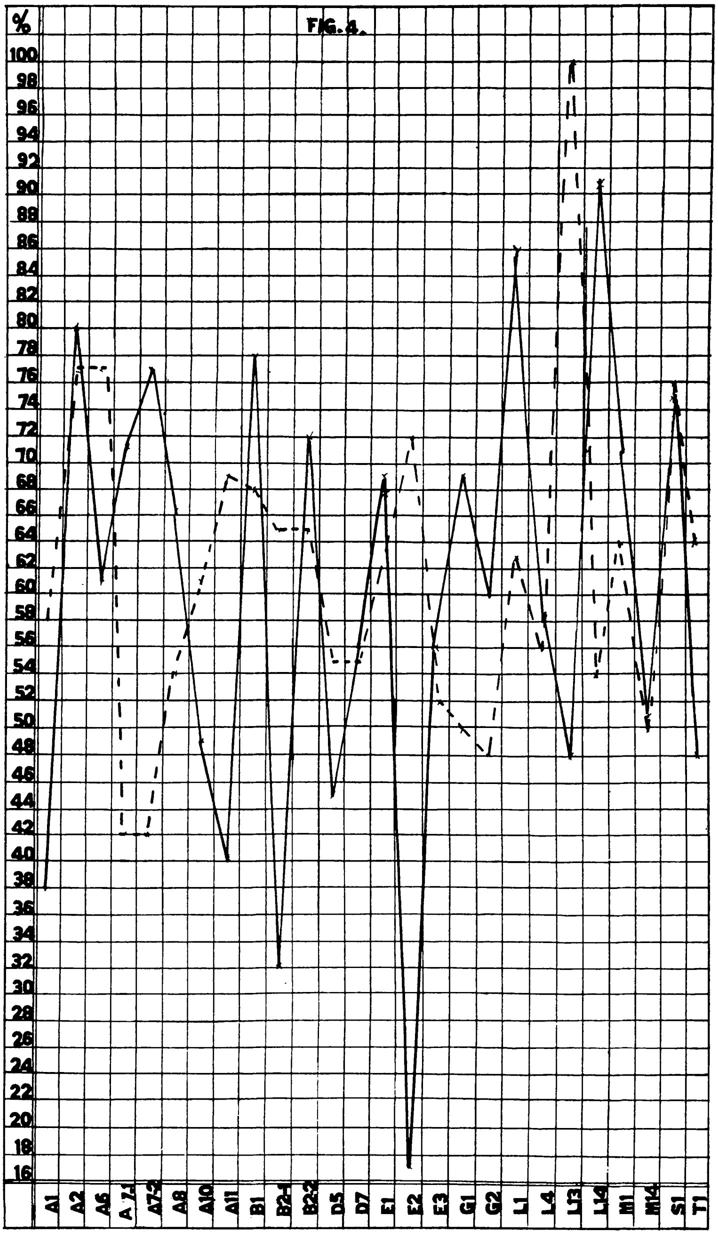

Fig. 4.—Solid line = percentage of fixative power of antiserums. Broken line = percentage of fixative power of antigens.

there was an inverse ratio between the two as suggested by Kinsella and Swift.[18] This inverse ratio was observed in only 38.4% of the immune serums and homologous antigens.

No other grouping of the positive fixations seemed possible from an examination of the whole table.

CONCLUSIONS

No correlation was observed between complement fixation tests and groups based on hemolysis and nonhemolysis. Nonhemolytic antiserums apparently gave even less specific complement fixation reactions than hemolytic antiserums.

There was, on the whole, only slight correlation between complement fixation tests and groups based on disease. The more definite disease groups, however, such as anterior poliomyelitis, erysipelas, and endocarditis tended toward group specificity.

There was no obvious relation between complement fixation tests and groups based on the fermentation of carbohydrates.

No inverse ratio was observed between the fixative power of an antigen and its antiserum.

The positive fixations could not be grouped in any way that would justify a classification of streptococci based on the complement fixation test.